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EXAMINER	
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Please find below and/or attached an Office communication concerning this application or proceeding.

PTO-90C (Rev. 10/03)

Office Action Summary	Application No. 10/661,790	Applicant(s) YAMAZAKI ET AL.	
	Examiner Christine Foster	Art Unit 1641	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on 29 July 2005.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-25,34-36 and 40 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-25,34-36 and 40 is/are rejected.

7)☒ Claim(s) 14 and 40 is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on 11 September 2003 is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

5) ☐ Notice of Informal Patent Application (PTO-152)

6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

Applicant's election without traverse of Group I, claims 1-25, 34-36 and 40 in the reply filed on July 29, 2005 is acknowledged. Applicant's cancellation of non-elected claims 26-33, 37-39, and 41 is acknowledged. Claims 1-25, 34-36 and 40 are currently pending.

Information Disclosure Statement

An Information Disclosure Statement (IDS) has not been received. The Examiner notes that submission of an IDS is not required, but reminds Applicant of the duty to disclose information material to patentability (see MPEP § 1.56).

The listing of references in the specification is not a proper information disclosure statement. 37 CFR 1.98(b) requires a list of all patents, publications, or other information submitted for consideration by the Office, and MPEP § 609 A(1) states, "the list may not be incorporated into the specification but must be submitted in a separate paper."

Oath/Declaration

The oath or declaration is defective. A new oath or declaration in compliance with 37 CFR 1.67(a) identifying this application by application number and filing date is required. See MPEP §§ 602.01 and 602.02.

The oath or declaration is defective because:
The specification to which the oath or declaration is directed has not been adequately identified. See MPEP § 602.

The oath filed in this application is a copy from that filed in prior provisional application 60/410,173, filed on September 11, 2002. However, in accordance with 37 CFR § 1.63, a

nonprovisional application requires an executed oath or declaration that refers to the specification being submitted. In particular, statements that the persons making the oath or declaration have reviewed and understand the contents of the application and acknowledge the duty to disclose information related to patentability must be made with regard to the document being submitted, except in the case of a continuation or divisional application (see also MPEP § 602). This is especially relevant in light of the additional material presented in the specification of the instant application, which is not present in the provisional application (e.g., Examples 6-7).

Drawings

Figures 1 and 3 should be designated by a legend such as --Prior Art-- because only that which is old is illustrated. See MPEP § 608.02(g). Figure 1 appears to be the same as Figure 1 of US Patent Nos. 6,228,326 and 6503452, and Figure 3 appears to be the same as Figure 4 of these patents.

The panels of Figure 5 appear to be too dark to interpret.

Corrected drawings in compliance with 37 CFR 1.121(d) are required in reply to the Office action to avoid abandonment of the application. The replacement sheet(s) should be labeled "Replacement Sheet" in the page header (as per 37 CFR 1.84(c)) so as not to obstruct any portion of the drawing figures. If the changes are not accepted by the examiner, the applicant will be notified and informed of any required corrective action in the next Office action. The objection to the drawings will not be held in abeyance.

Claim Objections

Claim 14 is objected to because of the following informalities: the claim does not terminate with a period.

Claim 40 reads "...the bulk aqueous phase further comprises a second test agent in and further comprising..." (lines 1-2). The placement of the preposition "in" appears to be an error. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

1. Claims 1-25, 34-36, and 40 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims lack a written description for the following reasons. Independent claim 1 is drawn to a method for assaying an interaction between a test agent and a lipid bilayer-associated component, where a physical property of one or more lipid bilayer expanses is evaluated during the method. The claims are therefore drawn to a genus of test agents and lipid bilayer-associate components. The claims also encompass detection of the interaction by evaluating a physical property of the bilayer, although other types of detection are also encompassed as there is no

recited correlation between evaluation and detection in the claim. In addition, a genus of possible physical properties which may be evaluated are encompassed, including those specifically claimed as listed in claim 8.

The claimed genus of test agents includes small molecules, cell surfaces, vesicles, and biomolecules, and other agents (see also claims 34-36). The claimed genus of lipid bilayer-associated components that interact with test agents include proteins, nucleic acids, glycolipids, lipopolysaccharide, sterols, lipid-linked molecules, fatty acids, and endotoxins (claims 2-3). However, the claimed genera of test agents and lipid bilayer-associated components have no disclosed partial structure, shared physical and/or chemical properties, or shared functional or other identifying characteristics. In particular, there is no disclosure of that the genera of test agents/lipid bilayer-associated components are known to affect physical properties of the bilayer upon interaction. With regard to claims 34-36, there is no disclosure that small molecules, proteins other than cholera toxin subunit B, cells, vesicles, phantom cells, liposomes, giant vesicles, lipid-covered glass beads, or components thereof are known to affect physical properties of the bilayer upon interaction.

The specification discloses only a single test agent (cholera toxin subunit B) that is capable, upon interaction with a lipid bilyaer-associated component (ganglioside GM1), of changing a physical property of the bilayer (membrane fluidity) (see p. 23, lines 9-12 in particular). There are no working examples of other test agent-component pairs, and there are no working examples of physical properties that are evaluated other than membrane fluidity.

With regard to claim 3, there is no written description of a method for detecting the interaction of a test agent with bilayer-associated bacterial endotoxin by evaluating a physical

property of the bilayer, as in claim 3. In Examples 3-4, 6 and in Figures 5C, 5D, 8, and 9, the endotoxin cholera toxin subunit B is the ***test agent***, and not the ***lipid bilayer-associated component*** (see p. 25, lines 17-19 in particular). Example 5 discloses endotoxins as lipid bilayer-associated components in which test agents are screened for interaction (p. 31, line 23 to p. 32, line 2). However, this example appears to be prophetic, and there is no disclosure that the interaction of such agents with bilayer-associated endotoxins affects membrane fluidity or other physical properties of the bilayer.

Claims 11-21 are drawn to evaluation of acyl chain mobility, membrane integrity, membrane appearance, and membrane continuity. The disclosure states that:

> In accordance with the present invention, binding events are detected through their effects on one or more physical properties of the lipid bilayer. These properties include, by way of example, but not limitation, membrane fluidity, acyl chain mobility, membrane integrity, membrane appearance, membrane continuity, membrane thickness, membrane bending modulus, and membrane tension. (p. 14, lines 13-18)

The claims additionally lack written description as there is no disclosure of binding pairs that are detected by evaluation of acyl chain mobility, membrane integrity, membrane appearance, or membrane continuity, and there are no working examples of detection of binding events by evalution of these parameters.

2. Claims 1-25, 34-36, and 40 rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for assaying an interaction between <u>test agents that are bacterial endotoxins</u> such as cholera toxin and <u>lipid bilayer-associated components that are endotoxin receptors</u> such as ganglioside GM1, does not reasonably provide enablement for assaying an interaction with other test agent-component pairs. The specification does not enable

any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Independent claim 1 recites a method for assaying an interaction between a test agent and a lipid bilayer-associated ligand. The ligand is associated with a substrate-supported lipid bilayer that is provided as part of a surface detector array device, which comprises multiple lipid bilayer expanses that are separated from each other by barrier regions. The method for assaying an interaction includes the step of contacting the device with the test agent, so as to allow the test agent to bind to its lipid bilayer-associated ligand. The method also includes the step of evaluating a physical property of one or more lipid bilayer expanses of the device.

The claims fail to recite that the step of evaluating a physical property is correlated with detection of the interaction between test agent and ligand (i.e., that the interaction is detected by evaluating a physical property). However, the disclosure states that "In accordance with the present invention, binding events are detected through their effects on one or more physical properties of the lipid bilayer (p. 14, lines 13-15). Because the claim does not specifically recite a detection step, traditional methods of detecting binding are encompassed by the claim, including secondary screening, detection of radiolabeled components, chemiluminescent detection, and others. The claim also encompasses the disclosed methods of detecting binding by evaluating bilayer properties. Test agents that are encompassed include small molecules, polypeptides, antibodies, biomolecules, cell surfaces, vesicles, and phantom cells, for example (see p. 3, lines 6-12). The claims are thus broadly drawn to methods of assaying binding or other types of interactions between a large number of possible test agents and lipid bilayer-associated

components, where the interaction may be detected by evaluating a physical property of the bilayer or by other detection methods.

The specification discloses that binding of cholera toxin to lipid bilayer-associated ganglioside GM1 can affect the fluidity of lipid molecules in the neighborhood of the ganglioside (p. 14, line 33 to p. 15, line 4). The specification also provides working examples demonstrating that binding of cholera toxin to membranes presenting GM1 may be detected indirectly by evaluating changes in membrane fluidity (Examples 3-4). The examples evaluate membrane fluidity by FRAP (Example 3) and by electrophoresis (Example 4).

The prior art teaches that the important feature in interaction of cholera toxin with ganglioside GM1 is polyvalent binding, as up to five GM1 receptors can bind to each cholera toxin molecule (Song et al., US Patent No. 6,297,059, column 8, lines 32-40 and column 6, lines 6-9). The interaction of cholera toxin with GM1 is therefore able to bring two or more GM1 receptors into close proximity (column 6, lines 1-5), which can be measured by fluorescence self-quenching or FRET (column 5, lines 49-51 and column 7, lines 44-67 in particular). Therefore, unlike the polyvalent cholera toxin, the binding of other test agents such as small molecules to bilayer-associated ligands would not necessarily have effects on membrane fluidity and other physical properties of the bilayer. In fact, the prior art teaches that some small molecules such as taurine have no effect on membrane fluidity upon interaction with membranes (Moran et al., "Effect of Tocopherol and Taurine on Membrane Fluidity of Retinal Rod Outer Segments," (1987) *Exp. Eye Res.* **45**:769-776, the abstract and p. 775, lines 10-12).

The specification provides no examples of test agents other than cholera toxin that affect membrane fluidity or other physical properties upon binding. There are no working examples of

other test agent/bilayer-associated component pairs that demonstrate such effects upon interaction.

The claims also encompass test agents interacting with lipid bilayer-associated integral membrane proteins. The prior art teaches that integral membrane proteins in supported bilayers may often be non-functional, and therefore incapable of interacting with test agents. Boxer et al. teach that:

> [I]ntegral membrane proteins are immobile or exhibit severely restriction motion in supported bilayers. It is presumed that this is a result of interactions between the membrane protein and the solid support; there have been relatively few careful studies of the functional consequences of this interaction, but it is generally thought that these interactions will reduce or eliminate protein function.

See Boxer et al., "Molecular transport and organization in supported lipid membranes" (2000) *Curr. Opin. Chem. Biol.* 4:704-9, p. 705, right column, "Softer surfaces and Tethering," lines 1-9). Boxer et al. further teach that "lipid bilayers and membrane proteins are notoriously difficult to work with" (ibid, p. 704, right column, lines 14-15). The instant specification discloses that "Observations of labeled CTB [cholera toxin subunit B] indicate that it is relatively immobile when bound to supported membranes" (p. 34, lines 21-22). From the above teachings of Boxer et al., this may indicate that CTB is non-functional, and would therefore be incapable of interacting with test agents. This would be of particular relevance to claim 3, in which bacterial endotoxins may be the lipid bilayer-associated component that interacts with test agents.

More generally, the specification does not provide direction regarding the preservation of function of integral membrane proteins or other lipid bilayer-associated components. There are no working examples of functional lipid bilayer-associated components that interact with test agents, other than ganglioside GM1.

In summary, the prior art establishes that the test agent-component pair of cholera toxin-ganglioside GM1 has important features (such as polyvalency) that allow for detection of interaction by changes in physical properties within the membrane. However these features are not shared by all test agent-component pairs encompassed by the claims. The prior art also teaches the unpredictability of preparing functional lipid bilayer-associated components such as integral membrane proteins. Therefore, due to the state of the prior art, the lack of direction/guidance presented in the specification regarding detection of interactions by evaluation of physical properties where the test agent-component pairs are other than cholera toxin-GM1, the lack of working examples directed to same, and the breadth of the claims, the specification fails to teach the skilled artisan how to make and use the claimed invention without undue experimentation.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-25, 34-36 and 40 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

3. Claim 1 is rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The preamble of the claim recites "assaying an interaction between a test agent and a lipid bilayer-associated component" (lines 1-2). However, the claim does not set forth steps for

assaying such an interaction. Furthermore, there is no recitation in the claim of a lipid bilayer-associated component.

4. Claim 1 is indefinite because it is unclear whether lines 7-8, which refer to a plurality of lipid bilayer expanses, are intended to be a method step or are reciting further components of the surface detector array device of line 3. The claim punctuation appears to indicate that lines 7-8 follow under the claim's preamble ("A method...comprising:"). However, lines 7-8 do not recite an active method step. For the purposes of examination the plurality of lipid bilayer expanses have been assumed to be further components of the surface detector array device as in Figure 1.

5. Claims 9-13 are indefinite because the scope of claims is unclear in light of the recited terms "membrane fluidity" and "acyl chain mobility". The terms are not defined in the specification, and both are disclosed as measurable by fluorescence anisotropy and fluorescence correlation spectroscopy (p. 26, lines 9-14 and p. 15, lines 5-10). It would seem that the terms are coextensive as increases in the mobility of the fatty acyl chains of the phospholipids in a membrane bilayer would necessarily be associated with increases in membrane fluidity.

6. Claims 17-18 are rejected as indefinite for recitation of "membrane appearance." The specification discloses methods by which "membrane appearance" may be evaluated (p. 16, line 33 to p. 17, line 10), but does not define the term.

7. Claim 36 is rejected for recitation of a "cell-vesicle." The term is not defined in the specification and does not appear to be well known in the art. It is unclear what is meant by the term.

8. Claim 36 is rejected for recitation of a "phantom cell." The term is not defined in the specification and does not appear to be well known in the art.

9. The term "giant" in claim 36 (referring to a “giant vesicle”) is a relative term that renders the claim indefinite. The term "giant" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

10. Claim 40 recites the limitation "the interaction of the test agent with the lipid bilayer-associated component" in lines 4-5. There is insufficient antecedent basis for this limitation in the claim.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

11. Claims 1-2, 4-8, 14-15, 19-20 and 34-36 are rejected under 35 U.S.C. 102(b) as being anticipated by Boxer et al. (US Patent No. 6,228,326; referred to as “Boxer et al.”), or, alternatively, by Boxer et al. (WO 98/23948; referred to as “Boxer ‘98”), which contains the same teachings. The column and line numbers discussed below refer to those in Boxer et al., US Patent No. 6,228,326, unless otherwise stated.

Boxer et al. teach a surface detector array device comprising a substrate having a surface defining a plurality of distinct bilayer-compatible surface regions separated by one or more bilayer barrier, where the bilayer-compatible surface regions and the bilayer barrier regions are formed of different materials. The surface array detector device also comprises a plurality of

lipid bilayer expanses located above the plurality of distinct bilayer-compatible surface regions, wherein the expanses are localized above the surface regions in the absence of covalent linkages to the surfaces and are separated from the surfaces by an aqueous film (column 3, lines 28-40). Boxer et al. further teach that the device may be used in a method for detecting binding between a test agent and a lipid bilayer-associated component (column 4, lines 25-31 and 41-43; column 12, lines 12-15, 31-33 and 39-42; and column 15, line 65 to column 16, line 7), wherein a bulk aqueous phase comprising the test agent is contacted with the device (column 17, line 65 to column 8, line 5). Supported bilayers may further comprise receptors including proteins or nucleic acids (column 4, lines 5-11 and column 12, lines 12-15 and 39-42 in particular). A physical property of a lipid bilayer expanse may be evaluated and correlated with binding of the test agent; for example, a change in the transmembrane voltage or current may be measured (column 18, lines 5-12) or changes in the bilayer environment surrounding a lipid bilayer-associated receptor in response to binding of a ligand may be detected by SPR (column 16, lines 11-38). With regard to claims 34-36, examples of test agents include the small molecule acetylcholine (column 18, lines 7-12), proteins (column 4, lines 5-9 and 26-31 and column 5, lines 16-30), and cells (column 18, lines 60-62).

With regard to claims 4 and 6-7, Boxer et al. teach a device comprising lipid bilayers doped with the fluorescently labeled lipid probe Texas Red DHPE (column 14, lines 46-47; column 20, lines 15-28; column 21, lines 16-22 and 52-55). The Texas Red label is attached to a lipid bilayer-associated component (phosphatidylcholine) that does not specifically bind to a test agent. With regard to claim 5, Boxer et al. teach attachment of hexa-histidine tags to ligands or receptors that are immobilized to the bilayer surface, as well as labels such as avidin or

streptavidin that may be coupled to biomolecules to link them to the supported bilayer (column 13, lines 36-59).

With regard to claims 8, 14-15 and 19-20, Boxer et al. teach measurement of membrane current (column 18, lines 5-12) in order to detect binding of acetylcholine to a surface array detector device comprising acetylcholine receptors. Boxer '98 additionally teaches analysis of capacitance and impedance (WO 98/23948, p. 22, lines 4-13). Although Boxer et al. and Boxer '98 do not specifically recite that membrane integrity and membrane continuity are evaluated, this would inherently be the case as the instant specification discloses that "membrane integrity may be evaluated by measuring…membrane current…" (p. 15, lines 21-25) and "membrane continuity may be evaluated by monitoring...membrane current…" (p. 16, lines 15-24).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

12. Claim 3 is rejected under 35 U.S.C. 103(a) as being unpatentable over Boxer et al. (US Patent No. 6,228,326; referred to as "Boxer et al."), or, alternatively, Boxer et al. (WO 98/23948; referred to as "Boxer '98") in view of Gutsmann et al. ("Interaction of CAP18-Derived Peptides with Membranes Made from Endotoxins or Phospholipids," (2001) *Biophysical Journal* **80**:2935-2945).

Boxer et al. and Boxer '98 are as discussed above, which fail to teach a method wherein at least one lipid bilayer expanse further comprises a bacterial endotoxin.

Gutsmann et al. teach a method for assaying an interaction between a test agent (CAP18-derived peptides) with differently composed lipid membranes, including bilayers made from bacterial endotoxin (lipopolysaccharide) (see the title and abstract and p. 2936, left column, second paragraph in particular). Gutsmann et al. teach that the reconstituted bilayers that comprise lipopolysaccharide mimic the outer membrane of Gram-negative bacteria (p. 2941, left column, lines 26-30) and such bilayers may be employed to assay interaction with the peptides by various biophysical techniques (p. 2941, left column, line 30 to right column, line 3 and the abstract, lines 11-12).

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the invention to employ bilayers comprising lipopolysaccharide, as taught by Gutsmann et al., in the method and device of Boxer et al. or Boxer '98, because Gutsmann et al. teach that such bilayers may be successfully employed in order to create a system that mimics the outer membrane of Gram-negative bacteria for use in methods to assay interaction of lipid bilayers with test agents, such as the methods of Boxer et al. and Boxer '98.

13. Claims 9-10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boxer et al. or, alternatively, Boxer '98 in view of Groves et al. ("Micropatterning Fluid Lipid Bilayers on Solid Supports," (1997) *Science* **275**:651-653).

Boxer et al. and Boxer '98 teach evaluation of membrane fluidity by FRAP and by electrophoresis, as discussed above. However, the references fail to teach such evaluation *in a*

method for assaying for an interaction between a test agent and a lipid bilayer-associated component.

Groves et al. teach evaluation of membrane fluidity in a surface array detector device by fluorescence recovery after photobleaching (FRAP) (see Figure 1 and p. 652, left column, lines 52 to middle column, line 13). Groves et al. further teach that such evaluation established that the membrane fluidity was long-range and that there was no intermixing between different corrals of the device (ibid and p. 651, right column, lines 6-16) and could also be used to determine effective barrier materials (p. 652, middle column, second paragraph).

Therefore, it would have been obvious to one of ordinary skill in the art to include the step of evaluating membrane fluidity by FRAP as taught by Groves et al. in the method of Boxer et al. or Boxer '98 in order to assess range of fluidity and/or measure intermixing between different corrals or expanses. One would have had reasonable expectation of success because Groves et al. teach that their surface detector array devices may be used in methods for assessing interaction with a test agent (see p. 653, middle column, paragraph 3 in particular), such as the methods of Boxer et al. and Boxer '98.

14. Claims 11-12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boxer et al. or, alternatively, Boxer '98 in view of Swamy et al. ("Spin-Label Studies on the Anchoring and Lipid-Protein Interactions of Avidin with *N*-Biotinylphosphatidylethanolamines in Lipid Bilayer Membranes" (1997) *Biochemistry* **36**:7403-7407).

Boxer et al. and Boxer '98 are as discussed above, which fail to teach methods wherein the physical property evaluated is acyl chain mobility or wherein acyl chain mobility is measured using an electron spin-labeled lipid.

Swamy et al. teach evaluation of acyl chain mobility in bilayer membranes using spin-labeled biotin-derivatized phosphatidylethanolamine ("biotin-PE") in order to investigate lipid-protein interactions, in this case interactions between avidin and biotin-PEs incorporated in membranes (p. 7403, left column, second paragraph, lines 1-5 and right column, second paragraph, lines 5-9). Swamy et al. further teach that the acyl chain mobility of the biotin-PE was selectively restricted upon binding to avidin (p. 7403, right column, second paragraph, lines 14-16).

Therefore, it would been obvious to one of ordinary skill in the art to evaluate acyl chain fluidity using a spin-labeled lipid as taught by Swamy et al. in the methods for assaying an interaction of Boxer et al. and Boxer '98, because Swamy et al. teach that such measurements may be used to study lipid-protein interactions. One would have had reasonable expectation of success in measuring using the spin-labeled lipids because Swamy et al. also teaches a method for assaying an interaction between a test agent (avidin) with a lipid bilayer-associated component (biotin-PE).

15. Claims 11 and 13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boxer et al. or, alternatively, Boxer '98 in view of Rooney et al. (Rooney, M.W., Lange, Y. and Kauffman, J.W. (1984) *J. Biol. Chem.* **259:**8281-8285).

Boxer et al. and Boxer '98 fail to teach a method wherein acyl chain mobility is evaluated, or wherein the mobility is evaluated by one of the techniques recited in claim 13.

Rooney et al. teach evaluation of membrane acyl chain mobility by FTIR in order to study the effects of interactions of a test agent (cholesterol) with the membrane (the abstract, lines 1-5; (p. 8281, right column, lines 1-6; p. 8282, left column, "Results and Discussion," lines

1-4). Rooney et al. found that acyl chain mobility was reduced in the presence of high amounts of cholesterol (p. 8281, right column, lines 1-6 and 13-18).

Therefore, it would have been obvious to one of ordinary skill in the art to include the step of evaluating membrane acyl chain mobility by FTIR as taught by Rooney in the method of Boxer et al. or Boxer '98, in order to study the effects of interactions of a test agent such as cholesterol. One would have reasonable expectation of success because Boxer et al. teach that a number of "suitable detection methods" may be employed in the method for assaying an interaction (column 18, line 4-12 and 34-38 in particular). Furthermore, the physical property that is evaluated by Boxer et al. (membrane current or voltage) is taught as being correlated with binding of the test agent (column 18, lines 7-12), while acyl chain mobility is taught by Rooney et al. as being correlated with such binding. Finally, one would also have reasonable expectation of success as both Rooney et al., Boxer et al. and Boxer '98 teach methods for assaying interactions of test agents with membranes.

16. Claims 16 and 21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boxer et al. or, alternatively, Boxer '98 in view of Grakoui et al. ("The immunological synapse: a molecular machine controlling T cell activation" (1999) *Science* **285**:221-227). As discussed above, Boxer et al. and Boxer '98 teach methods for assaying an interaction that comprise evaluation of membrane integrity and/or continuity by measurement of transmembrane voltage or current (column 18, lines 10-12). However, the references fail to specifically teach such evaluation by one of the methods recited in claims 16 and 21 in a method for assaying an interaction between a test agent and a lipid bilayer associated component.

Grakoui et al. teach a method for assaying an interaction between T cells and peptide-containing MHC molecules displayed on the surface of supported membranes, wherein fluorescence video microscopy was used to monitor the interaction (p. 221, right column, and Figure 1 in particular). Dynamic interactions were further probed by FRAP to assess the stability of complexes formed (p. 221, middle column, last paragraph, lines 3-9 in particular). MHC-peptides were free to diffuse in the supported bilayer (p. 221, right column, lines 19-25) and were observed to cluster together at the site of T cell synapse (see p. 225, right column, line 21 to p. 226, line 6 in particular). Although Grakoui et al. do not specifically recite that membrane integrity or continuity are evaluated, the instant specification discloses that "Membrane integrity may be evaluated by...using a method selected from the group consisting of...fluorescence recovery after photobleaching...[or] fluorescence microscopy" (p. 15, line 21 to p. 16, line 5) and that "Membrane continuity may be evaluated by...using a method selected from the group consisting of...fluorescence recovery after photobleaching" (p. 16, lines 15-24).

Therefore, it would have been obvious to include the step of using FRAP and/or fluorescence microscopy as taught by Grakoui in the methods of Boxer et al. or Boxer '98 in order to observe, monitor, and/or assess the stability of complexes formed between T cells and peptide-containing MHC molecules. One would have reasonable expectation of success because Grakoui et al. also teaches a method for assaying an interaction between a test agent (i.e., T cell) and a lipid bilayer-associated component (MHC-peptide) in a supported planar bilayer.

17. Claims 17-18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boxer et al. or, alternatively, Boxer '98 in view of Rinia et al. ("Visualization of Highly Ordered Striated

Domains Induced by Transmembrane Peptides in Supported Phosphatidylcholine Bilayers" (2000) *Biochemistry* **39**:5852-5858).

Boxer et al. and Boxer '98 fail to specifically teach evaluation of membrane appearance.

Rinia et al. teach evaluation of membrane appearance by direct imaging of phospholipid bilayers on a solid support by atomic force microscopy (AFM) in order to visualize lateral segregation of test agents (transmembrane model peptides) interacting with the bilayers (p. 5852, right column, lines 1-3 and p. 5853, left column, lines 26-35). Rinia et al. found that evaluation of membrane appearance by AFM was effective in visualizing aggregates of the test agents in the bilayer (p. 5857, right column, last paragraph) and further note that the AFM images produced may be useful in guiding computational studies of peptides or proteins in lipid bilayers, in particular studies of peptide-lipid aggregation (p. 5857, right column, second to last paragraph).

It would have been obvious to one of ordinary skill in the art to include the step of evaluating membrane appearance by AFM as taught by Rinia et al. in the method for assaying an interaction between a test agent and a lipid bilayer-associated component of Boxer et al. or Boxer '98 because Rinia et al. teach that AFM is useful in visualizing segregation or aggregation of peptides interacting with lipids in the bilayer. One would also be motivated to combine the teachings of Rinia et al. with the method of Boxer et al. or Boxer '98 in order to provide data for computational studies. One would have had reasonable expectation of success because Rinia et al. also teach a method for assaying an interaction between test agents (peptides) with lipid bilayer-associated components (lipids) on substrate-supported bilayers.

Claims 22-23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boxer et al. or, alternatively, Boxer '98 in view of Yang et al. ("New Approach for Atomic Force

Microscopy of Membrane Proteins" (1993) *Journal of Molecular Biology* **229**:286-290). Boxer et al. and Boxer '98 fail to specifically teach a method wherein membrane thickness is evaluated.

Yang et al. teach evaluation of membrane thickness by AFM in supported bilayers in order to study the three-dimensional interaction between cholera toxin with the lipid bilayer-associated ganglioside GM1 (the abstract, Table 1, p. 287, right column, Figure 3, and p. 289, right column, last paragraph in particular). Yang et al. also note that evaluation of membrane thickness and imaging by AFM may be applicable to other membrane proteins in supported bilayers (ibid).

Therefore, it would have been obvious to one of ordinary skill in the art to include the step of evaluating membrane thickness by AFM in the method of Boxer et al. or Boxer '98 in order to explore the three-dimensional interaction of the surface detector array device with a membrane protein. One would have reasonable expectation of success because like Boxer et al. and Boxer '98, Yang et al. also teach a method of assaying an interaction between a test agent (cholera toxin) and a lipid bilayer-associated component (GM1) in supported phospholipids bilayers.

18. Claims 24-25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boxer et al. or, alternatively, Boxer '98 in view of Hirn et al. ("The Effect of S-Layer Protein Adsorption and Crystallization on the Collective Motion of a Planar Lipid Bilayer Studied by Dynamic Light Scattering" (1999) *Biophysical Journal* 77:2066-2074). Boxer et al. and Boxer '98 fail to recite a method wherein membrane bending modulus or membrane tension are evaluated.

Hirn et al. teach a method for evaluating the effect of S-layer protein adsorption on the membrane bending energy and membrane tension of a planar lipid bilayer (the abstract). In

particular, Hirn et al. found that coupling of S-layer proteins to black lipid membranes caused a reduction in lateral tension (the abstract and p. 2071, left column, first two paragraphs). Large-scale crystallization after 24-h incubation with protein increased the bending modulus of black lipid membranes (p. 2073, right column, last paragraph, lines 1-5). Hirn et al. further teach that such measurements of membrane modes have the potential to be used to assess different stages of protein adsorption and recrystallization at a membrane surface (the abstract, last sentence) and can be used to study the detailed interaction mechanisms between the protein layer and the lipid membrane (p. 2067, lines 16-20).

Therefore, it would have been obvious to one of ordinary skill in the art to include the step of evaluating membrane bending modulus or membrane tension as taught by Hirn et al. in the methods of Boxer et al. and Boxer '98, because Hirn et al. teach that such properties change in response to protein adsorption to bilayers and that their measurement can be used to study the details of interactions of test agents (in this case proteins) with lipid membranes, which is the subject of the methods of Boxer et al. and Boxer '98.

19. Claim 40 is rejected under 35 U.S.C. 103(a) as being unpatentable over Boxer et al. or, alternatively, Boxer '98 in view of Keinanen et al. (US Patent No. 6,235,535).

Boxer et al. and Boxer '98 fail to teach a method wherein the bulk aqueous phase further comprises a second test agent and wherein the method determines whether the second test agent affects the interaction of the test agent with the lipid bilayer-associated component.

Keinanen et al. teach fluorescence-based immunoassay methods for detection of an analyte (the abstract), wherein two lipid-tagged antibody populations are attached to a lipid membrane (one population labeled with a FRET donor fluorophore and one population labeled

with a FRET acceptor fluorophore) (column 2, lines 50-60). In one embodiment, planar lipid membranes attached to a solid substrate are used (column 3, lines 4-13). When the lipid-attached antibody contacts a test agent (multivalent antigen), microaggregation occurs due to the free lateral mobility of the antibodies, which enables FRET (column 2, line 60 to column 3, line 4). Keinanen et al. also teach indirect immunoassay of a monovalent antigen, wherein a second test agent (monovalent hapten) is added in the presence of the first test agent (Ox16BSA multivalent antigen) (column 3, lines 30-41 and column 10, line 64 to column 11, line 4). Keinanen teach that addition of the second test agent affected the interaction of the first test agent with the lipid-attached antibody, as there was a decrease in the fluorescence changes proportional to the amount of the added second test agent (column 11, lines 4-9).

Therefore, it would have been obvious to one of ordinary skill in the art to include a second test agent, as taught by Keinanen et al., in the bulk aqueous phase comprising the test agent in the method of Boxer et al. or Boxer '98 in order to indirectly assay monovalent antigens. One would have reasonable expectation of success because Keinanen et al. also teaches methods involving interaction of test agents with planar lipid membrane-associated components.

Double Patenting

20. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970);and, *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground

provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

21. Claims 1-25, 34-36 and 40 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 22 of U.S. Patent No. 6,699,719 (Yamazaki et al.) in view of Boxer et al. (US Patent No. 6,228,326).

Although the conflicting claims are not identical, they are not patentably distinct because Yamazaki et al. also claim a method comprising assaying an interaction between a test agent and a lipid-bilayer associated component using a surface detection array device. The surface detection array device of Yamazaki et al. also comprises a substrate having a surface defining a plurality of distinct bilayer-compatible surface regions separated by one or more bilayer barrier regions that are formed of different materials, and wherein lipid bilayer expanses are localized above the bilayer-compatible surface regions. The method includes the step of contacting the device with a bulk aqueous phase comprising the test agent. The claimed method of Yamazaki et al. fails to recite the step of evaluating a physical property of lipid bilayer expanses.

However, Boxer et al. (as discussed above) teaches assaying an interaction between acetylcholine with a surface detection array device comprising acetylcholine receptor, wherein detection of binding is performed by evaluating a change in the current or voltage of the membrane (column 18, lines 5-2). One of ordinary skill in the art would recognize that the step of assaying an interaction as recited by Yamazaki et al. could comprise measurement of a physical property of the membrane (current or voltage) as in Boxer et al.

Conclusion

No claims are allowed.

The following is also cited by the examiner as prior art of relevance:

Song et al. (US Patent No. 6,297,059). Son et al., discussed in the USC 112, 1st paragraph rejection above, teaches a biosensor and methods for detection of multivalent target biomolecules such as cholera toxin. The biosensor comprises a fluid, substrate-supported bilayer membrane that includes a recognition molecule (such as ganglioside GM1) that is capable of binding with the target biomolecule. Binding is detected by fluorescence self-quenching or by fluorescence resonance energy transfer. However, Song et al. do not teach the particular surface detector array device of the instant invention.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christine Foster whose telephone number is (571) 272-8786. The examiner can normally be reached on M-F 8:30-5. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long Le can be reached at (571) 272-0823. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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